Citation:

Kilonzo-Nthenge A, Chen FC, Godwin SL. Occurrence of *Listeria* and *Enterobacteriaceae* in domestic refrigerators. *J Food Prot.* 2008; 71: 608-612.

PubMed ID: 18389708

Study Design:

Descriptive study

Class:

C - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

- To assess the overall microbial contamination on interior surfaces of domestic refrigerators
- To determine whether domestic refrigerators are a potential source of antibiotic-resistant bacteria.

Emphasis was placed on *Listeria* spp. and *Enterobacteriaceae* because of public food safety concern about these microorganisms.

Inclusion Criteria:

- Subjects from households that were part of a wider study in middle Tennessee that volunteered to participate in this study
- Participants on a list obtained from church groups and social service personnel in the area.

Exclusion Criteria:

- Subjects from households that were not part of the wider study, did not volunteer and did not live in middle Tennessee
- People not on a list obtained from church groups and social service personnel in the area.

Description of Study Protocol:

Recruitment

- Subjects in 137 households in middle Tennessee volunteered to participate in study, as part of a wider study
- A list of participants was obtained from church groups and social service personnel.

Design

Three swabs taken from interior (shelves, meat and vegetable drawers or middle drawer) of refrigerators:

- Approximately 400cm^2 were swabbed using sterile and moistened hydra sponges
- Sponges were transported to a laboratory in cooler and examined within two hours
- Butterfields phosphate buffer (25ml) was added to each sponge sample and pummeled in a Stomacher 400 Circulator at 230 rpm for two minutes
- Homogenate was used for analysis for *Listeria* spp., aerobic plate count and *Enterobacteriaceae* count.

Statistical Analysis

- Readings of aerobic colony counts (ACC) and *Enterobacteriaceae* counts (ETC) were converted to log CFU per sample before statistical analyses were performed
- The mean values were compared by the GLM procedure with SPSS 12.0 for Windows
- Differences were considered significant at $P<0.05$.

Data Collection Summary:

Timing of Measurements

Three swab samples (using sterile and moistened hydra sponges) were collected once from 137 household refrigerators and the sponges were transported back to a laboratory in a cooler and examined within two hours (design section includes protocol used to analyze samples).

Dependent Variables

- Isolation of *Listeria spp.*
- Aerobic plate counts
- *Enterobacteriaceae* counts.

How Measured

- Isolation and identification of *Listeria ssp:* 10ml of the homogenate from each sponge sample was transferred to 10ml of University of Vermont broth and incubated for 24 hours at 30°C; after primary enrichment, 1.0ml of University of Vermont broth was transferred to 10ml of secondary enrichment Fraser broth and incubated for 48 hours at 35°C; Fraser broth culture tubes showing blackening were streaked to *Listeria* selective agar plates with subsequent incubation for 48 hours at 35°C; following incubation, five typical colonies per plate were transferred onto tryptic soy agar with 6% yeast and incubated for 24 hours at 35°C. Presumptive *Listeria spp.* colonies were confirmed by Latex agglutination, Gram stain, oxidase test and catalyst test and identified by biochemical test strips
- Enumeration of *Enterobacteriaceae* and aerobic counts: Homogenized samples were serially diluted from 10 to one to 10 to five for subsequent plating on plate count agar and Petrifilm *Enterobacteriaceae*; plate count agar and Petrifilm plates were incubated for 48 and 24 hours at 35°C, respectively; typical colonies on Petrifilm were transferred to tryptic soy agar and incubated for 24 hours at 35°C. After incubation, three colonies (presumptive

Enterobacteriaceae) were isolated to make bacterial suspension to inoculate the API 20E strips. Oxidase tests and biochemical strips were used to identify isolates to the species or genus level
- Antimicrobial susceptibility testing: The Kirby-Bauer technique was used to determine sensitivity to the different antimicrobial agents; *Escherichia coli* 25922 was used as the quality control organism. Test isolates were grown with shaking in 5.0ml of Luria-Bertani broth at 37°C for 24 hours; each overnight culture was spread evenly onto Mueller-Hinton agar plate with cotton swab. Antibiotic disks were placed onto Mueller-Hinton plates and incubated at 37°C for 24 hours. The diameter of the zone around the disk was measured and interpreted according to the standard procedures outlined in the Clinical and Laboratory Standards Institute guidelines.

Description of Actual Data Sample:

- *Initial N:* 137 household refrigerators in middle Tennessee
- *Attrition (final N):*
  - 137 household refrigerators in middle Tennessee
  - Three samples from each refrigerator
  - Total number of samples=411
- *Location:* Middle Tennessee.

Summary of Results:

Key Findings

- *Listeria monocytogenes* was not isolated in any of domestic refrigerators sampled
- *Listeria innocua* (4.4%) was detected in meat drawers, vegetable bins and on the bottom shelves in some refrigerators
- *Enterobacter sakazakii* (2.2%) and *Yersinia enterocolitica* (0.7%) were isolated from the vegetable bins
- Additional species isolated included *K. pneumoniae* (23.4%), *Klebsiella oxytoca* (6.8%), *Klebsiella terrigena* (4.0%), *Enterobacter cloacae* (20.5%) and *Pantoea spp*. (13.9%)
- Regarding *Enterobacteriaceae* counts:
  - Highest *Enterobacteriaceae* counts (8.39 log CFU per sample) recorded were found in vegetable bins
  - Highest mean log CFU per sample count was in vegetable bins (3.00 + 0.18), followed by bottom shelves (2.38 + 0.21), middle shelves (2.19 + 0.5), meat drawers (1.53 + 0.22) and top shelves (1.09 + 0.37)
  - Mean count recovered from vegetable bins was significantly higher ($P<0.05$) than mean counts recovered from meat drawers and top shelves
  - *Enterobacteriaceae* counts on top shelves were significantly lower ($P<0.05$) than in the vegetable bins
- Regarding aerobic colony counts:
  - Aerobic colony counts ranged from 1.0 to 8.53 log CFU per sample
  - Highest counts (8.53 log CFU per sample) recorded were found in the vegetable bins
  - Mean log CFU per sample was highest in vegetable bins (5.38 + 0.12), followed by bottom shelves (5.01 + 0.15), middle shelves (4.48 + 0.13), meat drawers (4.42 + 0.15) and top shelves (3.74 + 0.33)
  - Mean log CFU per sample recovered from the vegetable bins was significantly higher

(P<0.05) than the mean count recovered from the bottom, middle, and top shelves and meat drawers
- Means from the bottom, middle, and top shelves and in meat drawers were not significantly different (P>0.05) from each other.

Sample Distribution of *Enterobacteriaceae* Counts from Domestic Refrigerators

(No. of Samples at log CFU/Sample) - (a)

	0-1.9	2.0-3.9	4.0-5.9	6.0-7.9	8.9-9.9	Total Samples
Top Shelf	15	5	2	0	0	22
Middle shelf	31	24	15	0	1	71
Meat/poultry drawer	43	24	5	2	0	74
Vegetable bin	43	41	42	10	0	136
Bottom shelf	45	33	25	5	0	108
Total	177	127	89	17	1	411

Sample Distribution of Aerobic Counts from Domestic Refrigerators

(No. of Samples at Log CFU/Sample) - (a)

	0-1.9	2.0-3.9	4.0-5.9	6.0-7.9	8.0-9.9	Total Samples
Top shelf	2	9	10	1	0	22
Middle shelf	0	28	38	4	1	71
Mean/poultry drawer	2	23	40	9	0	74
Vegetable bin	1	23	61	50	1	136
Bottom shelf	3	27	48	28	2	108
Total	8	110	197	92	4	411

Other Findings

Enterobacteriaceae antimicrobial resistance:

- Some *Enterobacteriaceae* isolates were intermediate and susceptible to selected antibiotics
- Percentage of resistant isolates was highest to erythromycin (39.9%), followed by ampicillin (33.8%), cefoxitin (12.8%), tetracycline (5%), streptomycin (4.1%), nalidixic acid (2.0%),

kanamycin (1.4%) and colistin (0.7%)
- None of the tested isolates were resistant to ciprofloxacin or gentamycin.

Author Conclusion:

- The presence of *L. innocua* in meat drawers, vegetable bins and on bottom shelves suggest favorable conditions for *L. monocytogenes* to grow and persist in domestic refrigerators
- *L. monocytogenes* persistence in ready-to-eat foods and its ability to grow at refrigeration temperatures indicates a public health concern
- Poor food handling practices may introduce *L. monocytogenes* in domestic refrigerators and, if allowed to persist, pose a significant risk to the consumer
- Because of the presence of *E. sakazakii* and *Y. enterocolitica* isolates in vegetable bins, it is strongly recommended that fresh produce be thoroughly washed before consumption
- *K. pneumoniae* and *E. cloacae* occurred frequently on the interior surfaces of domestic refrigerators. These microorganisms are present in the environment and gastrointestinal tracts of humans and animals, and thus are an indication of poor cleanliness and refrigerator management
- Most of the bacteria identified in the study are not usually associated with foodborne pathogens and are considered nonpathogenic to healthy adults
- Multi-drug resistance was found in *Klebsiella* spp., but not in *Y. enterocolitica* or *E. sakazakii*. These findings suggest that antibiotic-resistant *Klebsiella* is likely to be transmitted from contaminated poultry, beef products and fresh produce in domestic refrigerators to other parts of domestic kitchens
- Findings indicate the need for greater consumer education regarding proper domestic refrigerator cleaning and safe food handling practices in domestic kitchens.

Reviewer Comments:

- *Funding sources were not indicated*
- *No information was provided on demographics of households with refrigerators*
- *Because this study focused on diagnostics in the refrigerators of families, not on interventions applied to the family members themselves, ratings for elements two to five on the Research Design and Implementation Checklist were rated as "Not applicable."*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes

4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	N/A
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	N/A
2.2.	Were criteria applied equally to all study groups?	N/A
2.3.	Were health, demographics, and other characteristics of subjects described?	N/A
2.4.	Were the subjects/patients a representative sample of the relevant population?	N/A
3.	**Were study groups comparable?**	N/A
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	N/A
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	N/A
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	N/A
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	N/A

4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	N/A
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	N/A
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	N/A
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	N/A
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	N/A
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	Yes
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	N/A
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	N/A
6.8.	In diagnostic study, were details of test administration and replication sufficient?	Yes

7.	**Were outcomes clearly defined and the measurements valid and reliable?**		Yes
	7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
	7.2.	Were nutrition measures appropriate to question and outcomes of concern?	N/A
	7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	N/A
	7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
	7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
	7.6.	Were other factors accounted for (measured) that could affect outcomes?	???
	7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**		Yes
	8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
	8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
	8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
	8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
	8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	N/A
	8.6.	Was clinical significance as well as statistical significance reported?	Yes
	8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**		No
	9.1.	Is there a discussion of findings?	Yes
	9.2.	Are biases and study limitations identified and discussed?	No
10.	**Is bias due to study's funding or sponsorship unlikely?**		???
	10.1.	Were sources of funding and investigators' affiliations described?	No
	10.2.	Was the study free from apparent conflict of interest?	???